0022-202X/78/7006-0336$02.00/0
THE JOURNAL OF INVESTIGATIVE DERMATOLOGY, 70:336–341, 1978

Vol. 70, No. 6
Printed in U.S.A.

Glycosphingolipids and Ceramides in Human and Pig Epidermis

G. MAURICE GRAY, D.SC., AND RICHARD J. WHITE

MRC Unit on the Experimental Pathology of Skin, The Medical School, Birmingham, England

Total glycosphingolipids (glucosylceramides) were isolated from both pig (ear) and human (leg) epidermis and each separated into 4 distinct fractions of increasing polarity by silicic acid chromatography. Similarly, 4 separate ceramide fractions were obtained from each of the total free ceramides isolated from pig and human stratum corneum. All ceramide fractions contained large proportions (50% to 80% of total fatty acids) of C_{24} to C_{30} acids. The chromatographically more polar ceramides contained C_{24} to C_{30} 2-hydroxy fatty acids and in the most polar pig ceramide these accounted for 49% of the total fatty acids. Major bases in the pig ceramides were sphingenine, sphinganine, hexadeca-, heptadeca- and eicosasphingenine and the 2 most polar fractions also contained hydroxysphinganine. Human ceramide fractions were of similar base composition with nonadecasphingenine an additional major component. The least polar glucosylceramide fractions from both pig and human epidermis was a 1-(3′-O-acyl)-β-glucosylceramide. Linoleic acid was the major acid esterified to the glucose. The other glucosylceramide fractions contained fatty acids and bases whose compositions and distributions were similar to those in the ceramide fractions from stratum corneum. The glycosphingolipids and ceramides in the stratum corneum which contain high proportions of long chain (>C_{20}) saturated and 2-hydroxy fatty acids with high melting points (>75°) appear well-suited to withstand wide changes in temperature, ultraviolet radiation and atmospheric oxidation that may occur within the environment of the skin surface and, in the absence of phospholipids, are also sufficiently amphipathic to stabilize the lipid phase in the plasma membranes of the stratum corneum cell.

Previous work [1] has shown that gross changes occur in the lipid composition of epidermal cells in the course of their transit from the basal layer to the stratum corneum of mammalian epidermis. The fully differentiated cells in the stratum corneum have lost all of their phospholipids but retain their neutral lipids and much of their glycosphingolipid. One of the products of both phospholipid (sphingomyelin) and glycosphingolipid catabolism, ceramide, is also retained and is a major lipid component of the stratum corneum cell [1]. It was suggested [1] that, in the absence of phospholipid, the ceramide, glycosphingolipid, shown previously to consist entirely of glucosylceramides [2], and cholesterol sulfate may provide sufficient "polar" or amphipathic lipid in the plasma membrane of the stratum corneum cell to maintain a stable lipid phase in the classical bilayer form. To obtain further information on the "polarity" of the glucosylceramides and ceramides in pig and human epidermis we have examined their composition in detail and present our results in this paper.

MATERIALS AND METHODS

Reference methyl esters of saturated, unsaturated and 2-hydroxy fatty acids in the range C_{16} to C_{24} were obtained from Sigma Chemical Co. and/or Applied Science Laboratories, State College, Pa., U.S.A. DL-sphinganine and 4D-hydroxy-sphinganine were from Sigma Chemical Co. and 4-sphingenine from Koch Light, Colnbrook, Bucks., U.K. O-methylsphingosines were obtained by anhydrous acid methanolysis [3] of a sample of beef brain galactosylceramide. 1-β-glucosylceramide [4] and ceramide [5] were available from previous studies. 1-β-glucosyl-N-docosanoylsphinganine was a gift from Professor D. Shapiro (The Weizmann Institute, Israel). All solvents were of analytical grade and redistilled before use.

Analytical Techniques

Glucose in the glucosylceramides was identified and quantitatively estimated by gas chromatography on 3% OV-1 (support: Gas Chrom Q, 100–120 mesh, Applied Science Labs.) at 160°. Analysis was performed on the trimethylsilyl derivatives [6] of the methyl glucosides [7] with mannitol as an internal standard. Total long chain bases were determined by the method of Kisic and Rapport [8] with either 4-sphingenine or ceramide as the standard. Long chain bases obtained from the glucosylceramides and ceramides by acid hydrolysis [9] were converted to their trimethylsilyl derivatives [6] and separated by gas chromatography on 3% OV-1 at 220°. Identification of individual components was made by reference to standard compounds. Long chain bases were also identified by thin-layer chromatography on silica gel H (Merck, Darmstadt, W. Germany) with solvent system chloroform-methanol-4 M aqueous ammonia (80:30:3, by vol). Thin-layer plates were sprayed with an acetone solution (25 mg/100 ml) of fluorescamine [8] and bases were located with UV light. Fatty acids, as their methyl esters, were obtained from the glucosylceramides and ceramides by acid methanolysis [7] and analyzed by gas chromatography on 10% EGSS-X at 170° and 3% SE 30 at 220° (both phases on Gas Chrom Q, 100–120 mesh). The identity of 2-hydroxy fatty acids was confirmed by gas chromatography on 3% SE 30 of the fatty acid methyl esters before and after trimethylsilylation. Confirmatory evidence for the identity of unsaturated fatty acids was obtained by repeat gas chromatography of the methylesters, (a) after hydrogenation (platinum oxide catalyst) and (b) after bromination [10]. Proportions of individual long chain bases and fatty acids in the samples analyzed by gas-chromatography were calculated from the area under each component peak on the chromatograph recorder chart. Detector response (flame ionization) was linear for all compounds under the experimental conditions used.

Isolation of Ceramides From Pig and Human Epidermis and Stratum Corneum

Pig ears were obtained from an abattoir within 2 hr of death. Fresh, healthy human skin was obtained from amputated legs. Epidermis was obtained from pig and human skin and the total lipids were extracted as described previously [11]. Pig and human stratum corneum were obtained and the total lipids extracted as described previously [1]. The total lipids from each source, dissolved in hexane:diethylether (4:1 v/v) were put on to a column of silica gel 60 (230–400 mesh, Merck, Darmstadt; loading, epidermal lipids, 1 mg lipid phosphorus/gm; stratum corneum lipids, 20 mg lipid/gm) in the same solvent mixture. Solvent fractions were collected from the column and monitored by thin-layer chromatography on silica gel H (Merck) with light petroleum (b.p. 60–80°)/diethyl ether/acetic acid (30:70:1 by vol) as the solvent system. Lipids were detected by charring with 50% H_2SO_4 (v/v) [2]. After elution of cholesterol and fatty acids from the column the solvent was changed to hexane/diethyl ether (3:7 v/v) and a proportion of the ceramides was eluted from the column. The remainder were eluted with diethyl ether followed by chloroform:methanol (19:1 v/v). All fractions containing ceramides were bulked, the solvent was evaporated under vacuum and the lipids were redissolved in chloroform. Some fatty acids and a small amount of glycolipid were removed by chromatography on a column of silica gel 60 (230–400 mesh loading 10 mg lipid/gm) in chloroform. The fatty acids were eluted with chloroform and the ceramides with chloroform:methanol (19:v/v).

Manuscript received October 11, 1977; accepted for publication January 17, 1978.

Reprint requests to: Dr. G. M. Gray, MRC Skin Unit, The Medical School, Birmingham B15 2TJ, England.

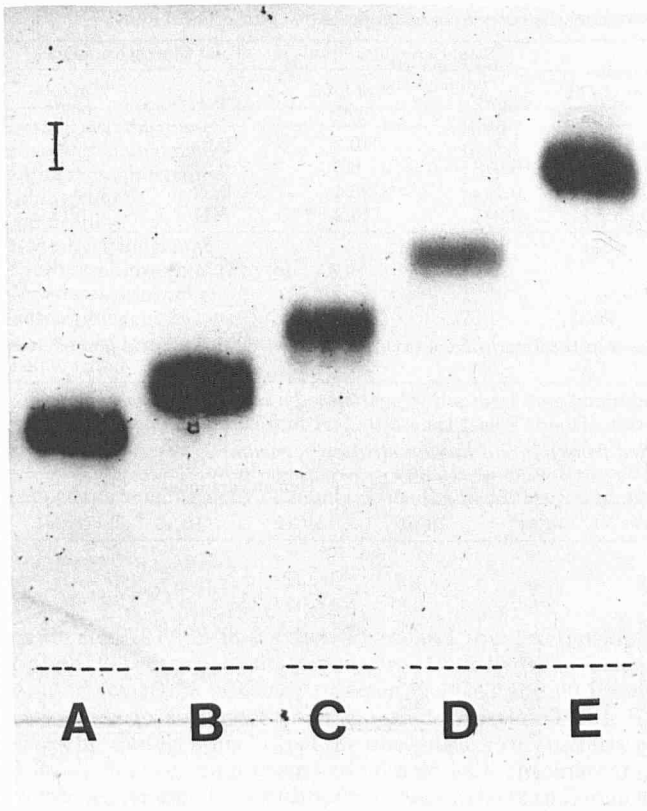

FIG 1. Thin-layer chromatographic properties of glucosylceramides isolated from human epidermis. Thin-layer adsorbent was silica gel H (0.25-mm thickness); solvent system, chloroform/methanol/water (40:10:1 by vol). Solvent was run to 17 cm from origin (*dotted lines*). *A*, glucosylceramide HGL4; *B*, glucosylceramide HGL3; *C*, glucosylceramide HGL2; *D*, 1-(3′-O-acyl)-β-glucosylceramide HGL1; *E*, reference marker, ceramide PCe4. *Vertical scale* mark equals 1 cm.

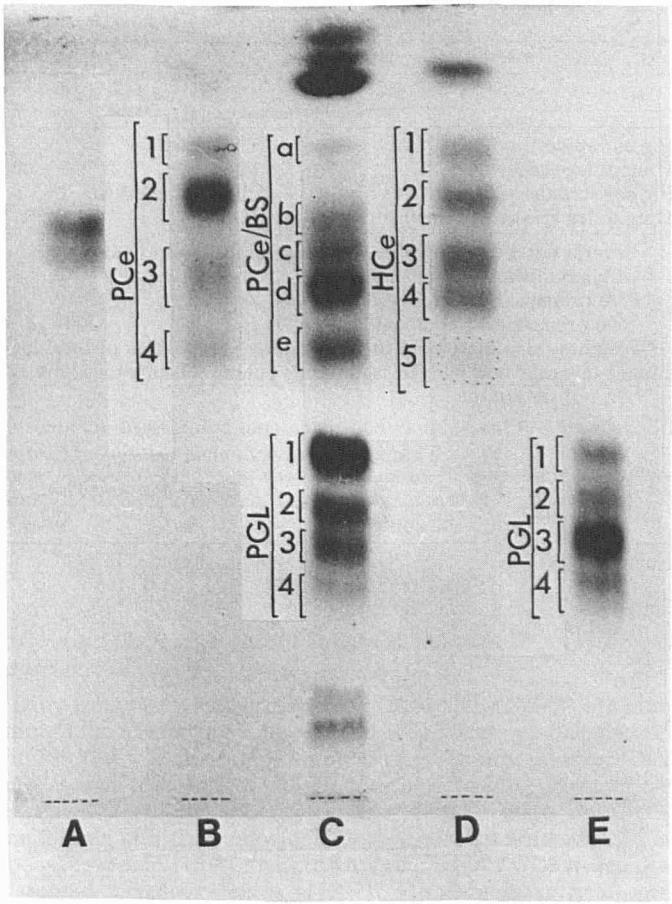

FIG 2. Thin-layer chromatographic separation of the glucosylceramides and ceramides from pig and human epidermis. Compounds were separated on silica gel H (0.25-mm thickness) with solvent systems (*a*) diethylether followed by (*b*) chloroform/methanol/water (40:10:1 by vol). *A*, reference ceramide (from pig lung (ref 5); *B*, total ceramides from pig stratum corneum separated into fractions, PCe1, 2, 3 and 4; *C*, total crude glucosylceramide and ceramide fraction from a mixed population of basal and spinous cells from pig epidermis. The ceramides were separated into fractions PCe/B5a, b, c, d and e and the glucosylceramides into fractions PGL1, 2, 3 and 4; *D*, total ceramides from human stratum corneum separated into fractions HCe1, 2, 3, 4 and 5; *E*, artificial mixture of pure glucosylceramide fractions PGL1, 2, 3 and 4 isolated from pig epidermis.

Samples of the total ceramides were examined by thin-layer chromatography on silica gel H. The thin-layer plate was developed successively in (a) diethyl ether and (b) chloroform/methanol/water (40:20:1, by vol) and lipids visualized by charring with 50% H_2SO_4 (v/v). Samples of the total ceramides were separated on thin-layer plates of silica gel H (pre-washed with chloroform/methanol/water, (5:5:1 by vol) and reactivated at 120° for 2 hr; loading approximately 5 mg lipid/plate) with the solvents (a) diethyl ether followed by (b) chloroform/methanol/water (90:10:1 by vol). Individual components were visualized by a brief exposure to iodine vapor and the appropriate areas of silica transferred to small glass columns. The ceramides were eluted with chloroform/methanol (3:1, v/v) and precipitated from hot methanol to remove traces of iodine. Each ceramide fraction was chromatographically pure. Recoveries of pig ceramides isolated as 4 separate fractions (PCe1, PCe2, PCe3 and PCe4) were in the range 80%to 85% of the total ceramides. The recoveries of 4 fractions (HCe1, HCe2, HCe3 and HCe4) isolated from the total ceramides of human stratum corneum were similar.

Isolation of Glucosylceramides From Pig and Human Epidermis

A portion of the total epidermal lipids, dissolved in chloroform was put on to a column of silica gel 60 (loading, 1 mg lipid phosphorus/gm) in chloroform and separated with the sequence of solvents described by Vance and Sweeley [12]. The glucosylceramides were eluted with chloroform/acetone (1:1, v/v) and acetone. They were slightly contaminated with ceramide, cholesterol sulfate and some unidentified sterol. Thin-layer chromatography on silica gel H in chloroform/methanol/water (40:20:1 by vol) separated the total glucosylceramides from both pig and human epidermis into 4 components (PGL1 to PGL4 and HGL1 to HGL4). Individual glucosylceramides were separated and isolated from the total mixture by thin-layer chromatography as described for the isolation of ceramides. Thin-layer plates (loading 5 mg lipid/plate) were developed with chloroform/methanol/water (40:20:1, by vol) and the glucosylceramides were recovered from the silica with chloroform/methanol (2:1 v/v). They were precipitated from a minimum quantity of chloroform/methanol (4:1, v/v) with excess ice-cold acetone, the precipitates washed with ice-cold acetone and redissolved in chloroform/methanol (4:1 v/v). Each glucosylceramide was chromatographically pure (Fig 1).

RESULTS

Composition of Ceramides and Glucosylceramides in Pig and Human Epidermis

Most of the ceramides in pig and human epidermis are located in the stratum corneum [1] and in practice we have found that the small quantities of ceramides present in the basal and spinous cells did not significantly affect the overall composition of ceramides from total epidermis and these were similar to, and representative of ceramides isolated only from stratum corneum. Amounts of ceramide in whole epidermal lipids and stratum corneum lipids are given in Table I. The total ceramides from either pig epidermis or stratum corneum were separated by thin-layer chromatography into 4 distinct components designated PCe1, (the least polar), PCe2, PCe3 and PCe4 (the most polar; see Fig 2). Their proportions in the total ceramides from pig stratum corneum were respectively 11%,

TABLE I. *Amounts of Ceramides and Glucosylceramides in epidermis and stratum corneum from pig and human skin*

	Tissue gm wt	Total Lipids gm wt	Total Ceramides		Total Glucosylceramides	
			gm wt[e]	% of total lipids	gm wt[e]	% of total lipids
Pig ear epidermis[a]	160 wet wt	13.8	1.4	10.2	0.6	4.3
Human leg epidermis[b]	32 wet wt	1.24	0.078	6.3	0.03	2.4
Pig ear stratum corneum[c]	5.2 freeze dried wt	0.54	0.11	20.4	0.02	3.7
Human leg stratum corneum[d]	ND[f]	0.29	0.047	16.2	ND	ND

[a] Several batches of pig ears were processed, total about 60 ears.
[b] Obtained from 5 amputated legs.
[c] Five preparations combined.
[d] Two preparations combined.
[e] Weights calculated from the quantitative estimation of total long-chain bases in total ceramides (average mol wt 623) and total glucosylceramides (average mol wt 800) isolated by column chromatography.
[f] ND, not determined.

TABLE II. *Fatty acid compositions of ceramides isolated from pig and human stratum corneum*

Fatty acid designation[e]	Pig ceramides						Human ceramides			
	PCe/BS[a]	PCe/SC[b]	PCe1[c]	PCe2	PCe3	PCe4[h]	HCe1[d]	HCe2	HCe3	HCe4
Normal series										
14:0	tr[f]	0.5[g]	1.0	tr	0.6	tr	1.3	tr		
15:0	tr	tr	0.9	tr	tr	tr	0.8	tr		
16:0	10.1	4.0	7.1	3.5	8.5	3.9	5.2	1.5	1.9	3.2
16:1	1.0	tr	1.8		0.7	1.4				
18:0	7.4	4.7	3.7	1.8	5.1	4.0	8.9	2.8	2.3	3.6
18:1	4.9	3.3	3.9	0.6	3.6	tr	9.2	4.7	2.2	1.1
18:2	4.3	4.2	24.0	tr	1.2	tr				
20:0	7.2	6.9	3.3	14.3	9.2	3.2	3.9	2.3	1.3	1.4
21:0	1.9	1.2	0.9	1.2	0.9	4.0	1.1	0.6	tr	0.9
22:0	6.0	8.9	5.1	9.5	6.8	2.7	6.1	5.0	2.9	2.9
23:0	1.8	1.8	1.6	2.5	1.1	0.6	3.2	3.5	2.8	2.1
24:0	14.2	17.8	10.1	27.1	14.3	3.2	15.8	26.6	22.1	8.9
25:0	1.7	2.7	2.3	3.5	2.2	1.2	8.4	9.9	9.2	14.0
26:0	10.7	13.4	9.4	15.2	10.9	3.6	9.7	22.7	21.3	9.6
27:0	2.1	1.6	1.4	2.0	0.9	0.8	4.4	4.1	4.0	10.9
28:0	8.5	14.3	13.3	15.0	10.7	3.9	4.4	11.3	9.1	3.3
29:0	2.0	1.0	1.1	1.2	1.1	tr		0.7	1.2	0.9
30:0	1.9	tr	4.9	2.6	0.8	tr		1.2	1.1	0.3
Hydroxy series										
20h:0					1.1	11.2	2.2			
22h:0									tr	1.1
24h:0	5.8	8.9	2.4		12.9	34.4	4.8		7.7	14.0
25h:0										5.3
26h:0	4.9	4.8	1.7		4.8	14.5	2.8		4.3	10.7
Unidentified	—	—	—		2.6	7.4	7.8	3.1	6.6	5.8

[a] PCe/BS, total ceramides isolated from 3 preparations (combined) of pig basal/spinous cells.
[b] PCe/SC, total ceramides isolated from pig stratum corneum (5 preparations combined).
[c] PCe1 to PCe4, chromatographically distinct fractions isolated from total ceramides of pig stratum corneum PCe/SC.
[d] HCe1 to HCe4, chromatographically distinct fractions isolated from total ceramides of human stratum corneum.
[e] Designation 14:0, long-chain saturated fatty acid with 14 carbon atoms; 16:1, long-chain unsaturated fatty acid with 16 carbon atoms and 1 double bond; 20h:0, long-chain saturated hydroxy fatty acid with 20 carbon atoms.
[f] Trace, tr < 0.5%.
[g] Values are expressed as a percentage of the total fatty acids.
[h] PCe4 also contained an unidentified fatty acid which could not be estimated by gas chromatography.

52%, 22% and 15% (estimated from long-chain base content [8]). The total ceramides from either human epidermis or stratum corneum were separated by thin-layer chromatography into 5 components designated HCe1 (the least polar), HCe2, HCe3, HCe4 and HCe5 (the most polar; see Fig 2). There was so little of HCe5 that recovery from the thin-layer chromatography plates was impracticable and was not pursued. The proportions of the 4 isolated components in the total ceramides was not quantitatively estimated but some indication of these can be obtained from thin-layer chromatography (Fig 2, *D*).

For comparative purposes the unfractionated total ceramides from pig stratum corneum (designated PCe/SC) and the total ceramides from the basal and spinous cells [1] of pig epidermis (designated PCe/BS) were also analyzed for fatty acids and long-chain bases. The amount of ceramides obtained from the basal and spinous cell preparations was small and the further isolation of chromatographically separable components of the total ceramides (see Fig 2, *C*) was not attempted.

The total glucosylceramides which were isolated from both pig and human epidermis were also separated each into 4 chromatographically distinct components (Fig 1 and Fig 2) designated PGL1 (the least polar), PGL2, PGL3 and PGL4 (the most polar) from pig and HGL1 (the least polar), HGL2, HGL3 and HGL4 (the most polar) from human epidermis. The structures of PGL1 and HGL1 were unusual and both had been identified previously [13] as 1-(3′-O-acyl)-β-glucosylceramide with a unique C_{35} unsaturated dihydroxy fatty acid attached to the long-chain base.

Fatty Acid Compositions of Ceramides From Pig Stratum Corneum (Table II)

The major acid (24% of total) in the least polar ceramide fraction, PCe1, was octadecadienoic acid (probably linoleic acid). 2-hydroxy acids accounted for a small proportion (4.1%) of the total acids. Tetracosanoic acid (lignoceric acid) was the

TABLE III. *Compositions of long-chain bases in ceramides isolated from pig and human stratum corneum*

	Long chain base designation[b]	Pig ceramides				Human ceramides			
		PCe1	PCe2	PCe3	PCe4	HCe1	HCe2	HCe3	HCe4
Hexadecasphingenine	16:1	14.4[a]	22.4	9.4	11.2	3.0	3.0	4.6	3.7
Hexadecasphinganine	16:0					3.2	2.3	1.2	1.7
Heptadecasphingenine	17:1	9.6	10.2	11.0	18.5	12.6	7.4	7.6	4.4
Sphingenine[c]	18:1	36.3	24.5	35.3	28.3	32.9	24.5	20.7	15.2
Sphinganine[c]	18:0	12.0	12.9	9.5	13.5	6.3	4.9	5.3	4.0
O-Methylsphingenine[c]	18(OMe):1	4.0	4.9	7.0	5.8	4.8	5.6	5.3	1.1
Hydroxysphinganine[c] (Phyto)	18h:0			7.3	11.6		2.2	8.0	23.1
Nonadecasphingenine	19:1					12.2	31.5	30.1	30.5
Eicosasphingenine	20:1	16.2	14.5	13.2	5.6	15.4	13.3	9.0	9.2
Docosasphingenine	22:1								
Unidentified		7.5	10.6	7.3	5.5	9.6	5.3	8.2	7.1

[a] Values are expressed as a percentage of the total long-chain bases.

[b] Designation similar to that for fatty acids (see Table II), thus 18:1 represents an unsaturated long-chain base with 18 carbon atoms and 1 double bond.

[c] With the exception of sphingenine, sphinganine, hydroxysphinganine and O-methylsphingenine the identification of long-chain bases was from gas chromatography data only. Thus the identification of other bases though probably correct, is regarded as tentative.

major acid (27.1%) in fraction PCe2 and this fraction did not contain 2-hydroxy acids. However, the 2-hydroxy acids accounted for 18.8% of the fatty acids in PCe3 and 60.1% of the fatty acids in the most polar ceramide fraction PCe4. Thin-layer chromatography [13] of the methylesters of the fatty acids of PCe4 showed the presence of an acid with chromatographic properties similar to the dihydroxypentatriacontadienoic acid ($C_{35}H_{66}O_4$) in glucosylceramide PGL1 [13]. This acid could not be identified or estimated by gas chromatography and was not included in the fatty acid composition of PCe4 (Table II). With the exception of PCe4 the ceramide fractions contained large proportions (41%–67%) of C_{24}-C_{30} normal saturated acids. The total unfractionated ceramides (PCe/SC) from stratum corneum contained the same range of fatty acids as the total ceramides (PCe/BS) from the basal and spinous cells but contained higher proportions of the C_{24}-C_{30} normal saturated acids and the 2-hydroxy acids.

Fatty Acid Composition of Ceramides From Human Stratum Corneum (Table II)

The thin-layer chromatographic properties of the least polar ceramide fraction HCe1 were identical to those of pig PCe1 (Fig 2) but its fatty acid composition was different. The major acid was tetracosanoic acid (15.8%) and octadecadienoic acid was absent. Tetracosanoic acid was also the major acid in fraction HCe2 (26.6%) and in HCe3 (22.1%). Both HCe1 and HCe3 contained 2-hydroxy acids but the most polar ceramide fraction HCe4 contained the largest proportion (31.1%) of these acids. All fractions contained a large proportion of C_{24} to C_{30} normal saturated acids (42%–76% of total acids).

Long Chain Bases in Ceramides From Pig and Human Stratum Corneum (Table III)

The predominant base in PCe1, PCe2, PCe3 and PCe4 was sphingenine. Other major bases were sphinganine, hexadeca-, heptadeca- and eicosasphingenines. PCe3 and PCe4 also contained hydroxysphinganine (phytosphinganine). Some minor components in all fractions were not identified. Sphingenine was the predominant base in the human ceramide fraction HCe1 but that in HCe2, HCe3 and HCe4 appeared from the gas chromatographic properties of its trimethylsilyl derivative to be nonadecasphingenine. This was not detected in pig ceramides. HCe2, HCe3 and HCe4 also contained, respectively, increasing proportions of hydroxysphinganine. Other components in all human ceramide fractions were sphinganine, hexadeca-, heptadeca- and eicosasphingenines and small proportions of hexadecasphinganine.

Fatty Acid Composition of Glucosylceramides From Pig Epidermis (Table IV)

Dihydroxypentatriacontadienoic acid ($C_{35}H_{66}O_4$) [13] accounted for half of the total acids in PGL1 and octadecadienoic acid (38.7%) was the major component in the remainder which were attached to C-3 of the glucose moiety [13]. Most of the acids (78.7%) in the glucosylceramide PGL2 were C_{24} to C_{30} compounds and the major acid was octacosanoic acid (27.3%). Docosanoic acid (15.7%) was the major acid in PGL3 which also contained 2-hydroxy acids (14.1%). The 2-hydroxy acids predominated (73.1%) in PGL4 the most polar glucosylceramide.

Fatty Acid Composition of Glucosylceramides From Human Epidermis (Table IV)

As in PGL1, the fatty acid attached to the base in HGL1 was dihydroxypentatriacontadienoic acid. Also octadecadienoic acid was the major acid (28.1%) attached to the glucose moiety of HGL1. Tetracosanoic acid was the major acid (26.4%) in HGL2 and hexacosanoic (21.3%) was the major acid in HGL3 which also contained 2-hydroxy acids (14.3%). Hexacosanoic acid (29.4%), octacosanoic acid (18.4%) and 2-hydroxy acids (15.4%) were predominant in HGL4 the most polar glucosylceramide from human epidermis.

Long Chain Bases in Glucosylceramides From Pig and Human Epidermis (Table V)

Sphingenine, sphinganine, hexadecasphingenine and heptadecasphingenine were present in all glucosylceramide fractions isolated from both pig and human epidermis and only fraction PGL4 from pig epidermis did not contain eicosasphingenine. Sphingenine was the major base (24.5% to 51.4% of total bases) in all fractions with the exception of PGL4 in which hydroxysphinganine predominated (30% of total bases). Hydroxysphinganine was also present in all other fractions with the exception of PGL1 and HGL1 the least polar fractions. Nonadecasphingenine was a major component of the bases in HGL2, HGL3 and HGL4 but was absent from all the fractions isolated from pig epidermis.

DISCUSSION

The total ceramides in pig and human stratum corneum are separated respectively into 4 and 5 groups of ceramides by thin-layer chromatography on silicic acid (Fig 2, *B* and *D*) because of differences in their polarities. Our results indicate that these differences are due partly to the amounts of 2-hydroxy fatty acids and/or long-chain bases that are present with additional

TABLE IV. *Fatty acid compositions of glucosylceramides isolated from pig and human epidermis*

Fatty acid Designation[a]	Pig glucosylceramides				Human glucosylceramides			
	PGL1	PGL2	PGL3	PGL4	HGL1	HGL2	HGL3	HGL4
Normal series								
14:0	—		1.2[e]	—	—	—	1.1	tr[d]
15:0	—		tr	—	—	—	tr	tr
16:0	3.0	3.2	4.9	1.2	5.4	9.1	5.7	1.9
16:1	1.0	tr	0.9	1.6	1.5	tr		
18:0	1.3	1.1	2.6	0.9	4.3	8.4	2.6	3.2
18:1	4.5	2.1	4.4		5.6	7.0	2.9	1.3
18:2	38.7		9.2		28.1	9.3		
20:0	1.5	5.0	15.7	2.5	5.1	5.2	1.5	1.3
21:0		tr	1.0					
22:0		4.8	6.0	2.2		3.4	7.7	3.1
22:1			1.5	tr				
23:0		1.2	2.0			1.2	4.8	1.1
24:0		17.0	12.8	2.1		26.4	17.1	3.9
24:1			1.7				1.5	1.6
25:0		3.3	2.6			3.1	6.0	4.3
26:0		19.5	8.1	11.3		18.2	21.3	29.4
27:0		3.5	0.6	1.6		1.1	3.6	7.8
28:0		27.3	4.2			1.2	3.1	18.4
29:0		2.3	1.0					
30:0		5.8	2.8			tr		
Hydroxy series								
20h:0			5.1	18.7				
22h:0			1.6	5.9				
24h:0			5.1	30.9			9.8	7.3
26h:0			2.3	17.6			3.5	8.1
Dihydroxy acid								
35(2h):2[c]	50	—			50			
Unidentified	—	3.9	3.7	2.8	—	6.6	7.8	7.3

[a] As for Table II.
[b] These fatty acids are esterified to the glucose in glucosylceramide PGL1 and HGL1 (ref 13).
[c] The amide-linked fatty acid in PGL1 and HGL1 was identified as dihydroxypentatriacontadienoic acid ($C_{35}H_{66}O_4$) (ref 13).
[d] tr, trace <0.5%
[e] Values are expressed as a percentage of the total fatty acids.

TABLE V. *Compositions of long-chain bases in glucosylceramides isolated from pig and human epidermis*

	Long-chain base designation[b]	Pig glucosylceramides				Human glucosylceramides			
		PGL1	PGL2	PGL3	PGL4	HGL1	HGL2	HGL3	HGL4
Hexadecasphingenine	16:1	4.4[a]	13.1	17.0	9.2	7.2	7.7	3.4	2.0
Hexadecasphinganine	16:0	2.1	2.8		5.6	3.3	2.0	3.2	2.0
Heptadecasphingenine	17:1	17.4	10.4	12.0	11.8	16.5	5.6	3.7	2.2
Sphingenine[c]	18:1	51.4	24.5	28.5	11.2	44.3	24.6	28.8	29.6
Sphinganine[c]	18:0	18.8	13.1	9.4	14.0	19.3	3.7	2.4	2.9
O-methylsphingenine[c]	18(OMe):1		2.8	2.5	4.4		5.4	6.7	9.0
Hydroxysphinganine[c] (Phyto)	18h:0		7.8	10.3	30.0		5.2	8.9	14.5
Nonadecasphingenine	19:1						21.3	19.0	26.2
Eicosasphingenine	20:1	4.4	19.1	14.3		3.1	19.8	10.9	6.6
Docosasphingenine	22:1		1.6	3.0					
Unidentified		2.0	4.8	2.5		6.3	4.9	13.0	5.1

[a, b] and [c] As for Table III.

hydroxyl groups. PCe4 has extra hydroxyl groups (Tables II and III) for the equivalent of 72% of the ceramide molecules whereas PCe3 has extra hydroxyl groups only equivalent to about 26% of the ceramide molecules. HCe4, which is chromatographically slightly more polar than PCe3 (Fig 2) has the equivalent of 54% of ceramide molecules with an additional hydroxyl group.

Though it is possible to form more than 200 different ceramides from the available fatty acids and long-chain bases in both pig and human epidermis, the range that the epidermal cell is able to synthesise, or requires, either as free ceramides, or as glucosylceramides or sphingomyelins is not known. There is only a small pool of free ceramides in the basal and spinous cells [1] of the epidermis and most ceramides are present in either glucosylceramides or sphingomyelins. The range of fatty acids in the total free ceramides of basal and spinous cells (Table II PCe/BS) is similar to that of the fatty acids in the ceramides from the stratum corneum (Table II, PCe/SC) but it is of interest to note that the distribution of groups of ceramides according to their polarity is significantly different (Fig 2, compare *B* to *C*). The ceramides in the basal and spinous cells may be a required component, albeit a minor one, of the membrane lipids or they may provide the precursor pool for the synthesis of both glucosylceramides and sphingomyelins. On the other hand the ceramides in the stratum corneum account for approximately 20% of the total lipids [1] in pig and human and are almost certainly products of the catabolism of sphingomyelins and glucosylceramides. It has been shown [14] that pig and human epidermis contain a sphingomyelinase which degrades sphingomyelin to ceramide and phosphorylcholine. One puzzling fact is that the fatty acids of pig and human epidermal sphingomyelins consistently contain tetracosaenoic acid (nervonic acid, C24:1) in significant amounts [2] but we have failed to identify this acid either in the total ceramides or

in the separated groups of ceramides from the stratum corneum. It may be that either it is enzymatically hydrogenated in the granular layer of the epidermis to tetracosanoic acid (lignoceric acid, C24:0) or it is released from the ceramide by a ceramidase [15] to be utilized by the epidermis for a specific purpose. Small amounts of tetracosaenoic acid have been reported [16] to be present in the total fatty acids isolated from the stratum corneum of human sole epidermis. We do not know whether the ceramides in the stratum corneum arise mainly from sphingomyelin metabolism or from glucosylceramide metabolism or equally from both. There are similarities between the fatty acid and base compositions of the ceramides PCe4 and the glucosylceramide PGL4; likewise for ceramides HCe3 and glucosylceramide is HGL3. The fatty acid and long-chain base compositions of pig and human epidermal ceramides are similar except for the presence of nonadecasphingenine in the human but not in the pig ceramides. Nonadecasphingenine has been reported in bovine milk [17], in rat and human brain and in human kidney [18].

Like the ceramides the glucosylceramides isolated from pig and human epidermis were separated into groups with different polarities (Fig 1 and Fig 2). The least polar group (PGL1 and HGL1, Tables IV and V) from both sources were recently identified as 1-(3′-O-acyl)-β-glucosylceramides [13] with a unique fatty acid, dihydroxypentatriacontadienoic acid ($C_{35}H_{66}O_4$) attached to the long-chain bases. This acid has not been identified in any other mammalian tissues and may only occur in the epidermis. The O-acylglucosylceramides are major components of the total glucosylceramides in both pig and human epidermis [13]. It is of interest that octadecadienoic acid (probably linoleic acid) is the major fatty acid linked to the glucose of the O-acylglucosylceramides. It has been suggested [19] that linoleic acid is important in maintaining the barrier function of the epidermis to control transepidermal water loss. As the barrier function of the epidermis appears to operate at the level of the stratum corneum, which does not contain phospholipids, it is possible that O-acylglucosylceramide may act as a major carrier for linoleic acid in the stratum corneum.

The most polar glycolipid fractions (PGL4, PGL3 and HGL4) contain considerable amounts of 2-hydroxy fatty acids and long-chain bases with an additional hydroxyl group and thus it is possible that some glucosylceramides have a total of 7 free hydroxyl groups in the molecule. The stability and structural integrity of the lipid phase in the membranes of mammalian cells depends on the amphipathic nature of many of the lipid components and in mammalian cells the major amphipaths are phospholipids. However, there are no phospholipids in the stratum corneum cells but they do contain ceramides and glucosylceramides [1]. It is reasonable to suppose that the glucosylceramides and possibly the more polar ceramides, especially those with hydroxy fatty acids and hydroxysphinganines, are a source of alternative amphipathic molecules able to fulfil the structural role of the catabolized phospholipids and maintain a stable lipid phase in the plasma membrane of the stratum corneum cell. It is also worth noting that the very long chain saturated fatty acids ($>C_{20}$) which account for the majority of the acids in both the glucosylceramides and ceramides have high melting points (>75°) and are stable to oxidation. Such properties are eminently suitable for cell components within the environment of the skin surface where they are exposed to, and must withstand, wide changes in temperature and ultraviolet radiation and atmospheric oxidation.

REFERENCES

1. Gray GM, Yardley HJ: Different populations of pig epidermal cells: Isolation and lipid composition. J Lipid Res 16:441–447, 1975
2. Gray GM, Yardley HJ: Lipid compositions of cells isolated from pig, human and rat epidermis. J Lipid Res 16:434–440, 1975
3. Coles L, Gray GM: Fluorimetric determination of sphingosine and its application to natural mixtures of glycosphingolipids. J Lipid Res 11:164–166, 1970
4. Adams EP, Gray GM: The structure of the oligosaccharide moieties of pig lung glycolipids. Chem Phys Lipids 1:368–375, 1967
5. Hay JB, Gray GM: The partial synthesis of some naturally occurring glycosphingolipids with special reference O-β-D-galactosyl-(1 → 4)-O-β-D-galactosyl-(1 → 1)-ceramide. Chem Phys Lipids 3:59–69, 1969
6. Carter HE, Gaver RC: Improved reagent for trimethylsilylation of sphingolipid bases. J Lipid Res 8:391–395, 1967
7. Sweeley CC, Vance DE: Lipid chromatographic analysis, Vol 1, first edition. Edited by GV Marinetti. New York, Marcel Dekker, 1967, pp 465–495
8. Kisic A, Rapport MM: Determination of long chain base in glycosphingolipids with fluorescamine. J Lipid Res 15:179–180, 1974
9. Gaver RC, Sweeley CC: Methods for methanolysis of sphingolipids and direct determination of long chain bases by gas chromatography. J Am Oil Chem Soc 42:294–298, 1965
10. James AT, Martin AJP: Gas-liquid chromatography: The separation and identification of the methyl esters of saturated and unsaturated acids from formic acid to n-octadecanoic acid. Biochem J 63:144–152, 1956
11. Gray GM: Phosphatidyl-(N-acyl)-ethanolamine a lipid component of mammalian epidermis. Biochim Biophys Acta 431:1–8, 1976
12. Vance DE, Sweeley CC: Quantitative determination of the neutral glycosylceramides in human blood. J Lipid Res 8:621–630, 1967
13. Gray GM, White RJ, Majer JR: 1-(3′-O-acyl)-β-glucosyl-N-dihydroxypentatriacontadienoylsphingosine, a major component of the glucosylceramides of pig and human epidermis. Biochim Biophys Acta, 528:127–137, 1978
14. Bowser P, Gray GM: Sphingomyelinase in pig and human epidermis. J Invest Dermatol, this issue
15. Gatt S: Enzymatic hydrolysis of sphingolipids. 1. Hydrolysis and synthesis of ceramides by an enzyme from rat brain. J Biol Chem 241:3724–3730, 1966
16. Ansari MNA, Nicolaides N, Fu HC: Fatty acid composition of the living layer and stratum corneum lipids of human sole skin epidermis. Lipids 5:838–845, 1970
17. Morrison WR: Polar lipids in bovine milk. 1. Long-chain bases in sphingomyelin. Biochim Biophys Acta 176:537–546, 1969
18. Karlsson K-A, Holm GAL: Studies on sphingosines. 9. C_{19}-Sphingosines, hitherto unknown sphingosines. Acta Chem Scand 19:2423–2425, 1965
19. Prottey C: Investigation of functions of essential fatty acids in the skin. Br J Dermatol 97:29–38, 1977